scope of the claims. Applicants respectfully submit, however, that these amendments are entered solely to abide by the established Patent Office policy as set forth at 1077 OG 24, April 21, 1987, and do not constitute an admission that such claimed subject matter would not otherwise be enabled and patentable based on the present disclosure. These amendments introduce no new matter.

Claims 33 and 34 have also been amended to more clearly point out that the claimed differentiated cells are isolated, and may be distinguished from transformed cells growing *in vitro*. The first part of this amendment was suggested by the Examiner on page 6 of the Office Action. These amendments introduce no new matter.

Claims 17-22, 25-27, 29, 31, 33, 34, 57, 59, 61, 64, 66, 68, 71 and 72 have also been amended to clearly point out that such embryos, fetuses, offspring, progeny and cell lines may be distinguished from other similar products made by different methods on the basis of a unique property of their genotype. This amendment merely points to the inherent novelty of the disclosed invention and presents no new matter.

Finally, new Claims 78 and 79 have been added which more clearly define the differentiated cells of Claims 33 and 34 as being produced following a process of making a CICM cell line wherein this process involved cell fusion. Support for the new

claims may be found in the specification at the bottom of page 25. Claims 1-34 and 55-79 are pending.

On page 2 of the Office Action, Claims 17-22, 25-27, 34, 57, 59, 61, 64, 66, 68, 71 and 72 have been rejected under 35 U.S.C. §101 as being directed to non-statutory subject matter because they encompass human embryos, fetuses, offspring and progeny. As discussed above, claims directed to embryos, fetuses, offspring and progeny have now been amended to clarify that the scope of such claims is limited to non-human mammals pursuant to the Notice published in the Official Gazette at 1077 OG 24, April 21, 1987. Accordingly, the rejection of the above claims under 35 U.S.C. §101 has been rendered moot, and should now be withdrawn.

Claim 34, on the other hand, is directed to human differentiated cells. Applicants assume that this claim has been rejected under 35 U.S.C. §101 as a result of the clarity issue arising under 35 U.S.C. §112, second paragraph, as set forth on page 6 of the Office Action. Specifically, the Office Action indicated that it was not clear whether Applicants are claiming cells in a human, or the human itself, presumably as a conglomeration of differentiated cells. Applicants respectfully submit that claim 34 is directed to neither cells in a human, nor to a human itself, and have amended the claim to more clearly point out that the claimed cells are isolated. Claim 33, also rejected under 35 U.S.C. §112, second paragraph for the same reasons, has likewise been amended. Accordingly, Applicants respectfully request that

the rejections of Claim 34 under 35 U.S.C. §101 and §112, second paragraphs, and the rejection of Claim 33 under 35 U.S.C. §112, second paragraph, be reconsidered and withdrawn in view of the proposed amendments.

Beginning on page 2 of the Office Action, all pending claims have been rejected under 35 U.S.C. 112, first paragraph as allegedly containing subject matter that was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. Applicants respectfully traverse.

The main basis of the rejection appears to be that the "nature of the art at the time of filing was such that the cloning of a mammal... from a fully differentiated cell or from a nucleus of a fully differentiated cell was unlikely to be successful." (Specification, page 3, first full paragraph; emphasis added). In fact, the Examiner admits in the Office Action at page 3 (same paragraph) that at the time of filing, while the art supplied guidance "on the production of mammals by the insertion of a nucleus from a totipotent embryonic cell into an enucleated oocyte, the artisan could not have found such guidance when the nucleus was from a fully differentiated cell. For this the artisan *could only rely on the instant specification*." (Emphasis added for later referral.)

The Office Action goes on to stress that, while the specification provides working examples to show the development

of chimeric and transgenic embryos and fetuses, it does not disclose the production of a live birth. In this regard, Applicants are pleased to present evidence pursuant to 37 CFR §1.132 in the attached Declaration (unexecuted) by Steven L. Stice, Ph.D., first-named inventor of the present invention. (The executed declaration will be promptly submitted upon receipt by Applicants' representative.) In this Declaration, Applicants now introduce evidence that seven live calves have been produced using a donor nucleus from a differentiated cell according to the method of the present invention.

Indeed, as discussed in the Declaration, seven live, transgenic, "normal and vigorous" calves have been produced from seven of twelve recipient females implanted with an NT unit made according to the present invention. This represents a success rate of over 50%, which strongly suggests that the disclosed method is both predictable and successful when the nucleus from a differentiated cell is used. The Examiner is further respectfully advised that these successes have received tremendous attention by the press. In fact, these results have been reported by all the major television networks and newspapers. In support thereof, Applicants provide for the Examiner's consideration a copy of an article that appeared in *USA Today*, which is representative of the numerous press releases relating to the subject cloning technique, and the efficacy thereof (as evidenced by the birth of live transgenic bovines).

The Office Action also comments at page 4 that "even post-filing art, employing a [similar method] resulted in the birth of only one sheep, further demonstrating the unpredictability of the claimed method." However, as presented in the attached Declaration, Applicants have produced seven cloned transgenic animals using the disclosed method. "The mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it." *Gould v. Quigg*, 3 USPQ 2d 1302, 1304 (Fed. Cir. 1987). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, as it relates to claims directed to methods of making NT (nuclear transfer) units using the nucleus from a differentiated cell.

The Office Action also maintains at the bottom of page 4 that "the production of chimeric animals is of itself lacking reproducibility." Applicants are pleased to present evidence in the attached unexecuted Declaration of Applicants' recent success in this regard.

As detailed in the attached paper recently submitted to Nature Medicine, Applicants have recently had success in producing chimeric calves from both transgenic embryo-derived ES-like cells, and transgenic NT-derived ES-like cells (see pages 6-7 of attached paper). In particular, six calves were born from chimeric embryos that were generated using NT-derived ES-like cells

wherein the donor cell nucleus was derived from differentiated bovine fibroblasts obtained from a 60-day fetus (see the bottom of page 5). Nine of eleven calves born had multiple tissues that were positive for β-Galactosidase expression (see Figure 3), including the gonadal tissue. These results strongly suggest that production of chimeric animals can be repeated by one of skill in this art with a high degree of predictability and relatively certain chance of success.

With regard to chimeric animals, the Office Action also states that there "are not guidelines provided in the specification for the production of a chimeric mammal such that the mammal has a use to the art" (page 4, first full paragraph). However, Applicants respectfully refer first to the decision of the Federal Circuit in *Hybritech, Inc. v. Monoclonal Antibodies, Ind.*, 231 USPQ 81, 94 (1986), where the court made it clear that "A patent need not teach, and preferably omits, what is well known in the art."

Next, Applicants respectfully point out that the Patent & Trademark Office has recently recognized that methodology involved in making and using chimeric mammals by combining an ES cell with an embryonic blastocyst is standard practice in the art. For instance, in U.S. Patent No. 5,690,926 (attached for the Examiner's convenience), claims have recently issued which are broadly directed to a method of making chimeric animals using an established embryonic cell line (see claims 1, 4 and 5). As stated in

the disclosure of this patent (col. 7, l. 33-37), "chimeric animals can subsequently be bred to obtain germ line transmission of ES cell traits... [and] methods of producing such chimeric animals are well established (Robertson (1987))."

Hence, according to U.S. Patent 5,690,926, production and use of chimeric animals from established ES cell lines has been standard practice in the art since at least 1987, which is well before the filing date of the present invention. Furthermore, Applicants respectfully point out that the claims in question are directed to methods of making chimeric embryos using the NT units of the present invention. Indeed, "where the... compositions claimed by applicants are known *and their utility... is known*, the Examiner is incorrect in rejecting claims reciting such... compositions as being based on an insufficient disclosure, *particularly when applicant's invention does not reside in the use of any particular [composition]*. *Ex Parte Gleixner*, 214 USPQ 297, 298 (PTO Bd App 1980) (Emphasis added).

Because methods of making chimeric animals are known in the art, and the utility of such animals is known in the art, Applicants' disclosure need only demonstrate stem cells which are amenable to a method of making such animals. Applicants have clearly demonstrated this in the disclosure of NT units made with the nucleus of a differentiated cell. Therefore reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first para-

graph as it pertains to making chimeric animals is respectfully requested.

The Office Action goes on to comment on page 5 that "the showing of β-galactosidase expression is not sufficient to demonstrate... expression sufficient for transplantation", and that "it is not clear how a mammal that does not express a transgene that alters the host-graft response will be useful as an organ donor." The Office Action then questions the intended use of the chimeric or transgenic mammals of the present invention.

In response, Applicants first respectfully point out that claims 33, and particularly claim 34, are directed to and encompass human isolated differentiated cells derived from CICM cell lines, which are in turn derived from the NT units of the present invention. Because the nucleus of an adult differentiated cell could be used to make such cell lines, a differentiated cell line could be made which has the identical genotype as an adult human. Such cells could be used directly for transplantation into a human without the need for a transgene directed to deterring graft rejection. This is just one example of how the NT units, and cell lines derived from such units could be used for human therapies without relying on transgene expression to prevent graft rejection.

Secondly, Applicants respectfully point out that the methods of the present invention make the use of heterologous genes as transgenes more predictable and reliable, since a nuclear donor may be selected which is transfected beforehand,

and has already been shown to demonstrate sufficient transgene expression. This benefit is clearly discussed in the specification beginning on page 15, line 23, where it is stated

> The present invention allows *simplification* of the transgenic procedures by working with a differentiated cell source that can be clonally propagated. This eliminates the need to maintain cells in an undifferentiated state, thus genetic modifications, both random integration and gene targeting, are more easily accomplished. Also, by combining nuclear transfer with the ability to modify and select for these cells in vitro, this procedure *is more efficient than previous transgenic embryo techniques*. (With emphasis.)

Thus, the concern discussed in the Office Action that a transgene would need to be expressed "sufficiently" does not raise an issue with regard to unpredictability because the methods of the present invention are a simplification over the methods of the prior art. Indeed, a differentiated cell having a genotype of interest may be transfected with a gene of interest before nuclear transfer, and selection of a cell containing a nucleus from which the transfected gene is sufficiently expressed may be accomplished without using cytokines, and without worrying about differentiation of the cells. The method of the present invention therefore removes the unpredictability element from the expression of transgenes in transgenic animals, so it is not clear why the Office Action considers this element, in particular, to be unpredictable. Transgenic animals expressing genes at an adequate level are fully enabled by the present disclosure, and of course such genes could be chosen for the purpose of preventing or deterring the rejection of transplants.

As far as other uses of the claimed mammals is concerned, it is not clear how the Office Action can assert that uses of transgenic animals are "not apparent" given the extreme competition in the art to produce such animals. "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent *coupled with information known in the art.*" *U.S. v. Telectronics, Inc.*, 8 USPQ 2d 1217, 1223 (Fed. Cir. 1988) (With emphasis.) Because the "use" prong of 35 U.S.C. §112, first paragraph, is also evaluated in consideration of what is known in the art, one need only look at any of the multitude of references currently available discussing the potential uses of transgenic animals.

For example, Hyttinen et al., cited in the Office Action, discusses the potential uses of transgenic animals in the first paragraph of page 606. As discussed in the reference, transgenic farm animals, including cows and sheep, "have become an attractive alternative to microbial and animal cell bioreactors... The production of substantial amounts of human hemoglobin has been accomplished with the aid of transgenic pigs." The uses of such animals, therefore, are well known in the art, and are virtually unlimited. The problem, on the other hand, as also discussed in Hyttinen, is the production of large amounts of transgene-positive embryos for subsequent production of such animals. The present invention solves this problem, and the disclosure is therefore fully enabled when "coupled with information known in

the art." *Telectronics, supra*. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, as it pertains to the production and use of transgenic animals is respectfully requested.

Claims 17-19, 25-27, 71-73, 75 and 77, and Claims 20-22, 57, 59, 61, 64, 66, 68, 74 and 76 have been rejected under 35 U.S.C. §102(b) as being anticipated by U.S. Patent No. 5,057420 and Hyttinen et al., respectively. In addition, Claim 29 has been rejected under 35 U.S.C. §102(b) as being anticipated by Sims et al. Because the instant claims are product by process claims, the Office Action maintains that the cited references clearly anticipate the claimed embryos, fetuses, offspring, and progeny, and chimeric or transgenic embryos, fetuses, offspring and progeny, and CICM cell line, in the absence of some distinction which indicates a "structural or functional difference" between the claimed products and those of the prior art. Applicants respectfully traverse, and can readily identify a structural distinction.

Because the claimed embryos, fetuses, offspring, progeny and cell line are made from an NT unit which is in turn made <u>using the nucleus from a differentiated cell</u>, the claimed embryos, fetuses, offspring, progeny and cell line of the present invention may be distinguished from similar products in the prior art in that they have <u>the identical genotype</u> as a <u>differentiated</u> cell, fetus or mammal of the same species <u>in existence prior to</u>

nuclear transplantation, wherein such prior existing cell, fetus or mammal was not formed using nuclear transfer techniques. The probability of this happening purely by chance with any other embryo, fetus, offspring or progeny is virtually zero.

"Differentiated" cells are defined in the specification at the bottom of page 19 as any cells past early embryonic stage, or those from at least the embryonic disc stage (day 10). Neither the `420 patent, nor Hyttinen, nor Sims teaches embryos, fetuses, offspring and progeny, or ICM cell lines, that were generated from such a differentiated cell. Indeed, although the `420 patent uses nuclear transplantation to create embryos, as stated in col. 3, l. 29-30, the donor nucleus is obtained from a 16 to 32 cell stage embryo which is typically collected on day 5. Thus, while the method of the `420 patent may result in cloned mammals having the identical genotype if more than one cell from the day 5 embryo is used for nuclear transfer, the method according to the `420 patent could never result in embryos, fetuses, offspring or progeny that have the identical genotype of a prior-existing differentiated cell or mammal that was not made using such techniques.

The Hyttinen et al. reference, on the other hand, does not concern nuclear transfer and does not even concern the production of chimeric or transgenic animals using a cloned ES cell line. Rather, Hyttinen generated transgenic embryos and calves by micro-injecting a gene construct into an oocyte that had been

fertilized *in vitro* (see the abstract). Thus, each of these transgenic embryos was genetically distinct from, not only the parent cows, but the other embryos as well.

Finally, Sims et al. do teach the culture of an ICM cell line. However, this cell line was started from an oocyte that was fertilized by the sperm of one of five bulls (see the bottom of page 6143 to the top of page 6144). As such, it does not have the identical genotype as either of the cows used to produce it. Furthermore, while calves then generated using nuclei transfer from such a cell line would be genetically identical to each other, none of these calves will have a genotype identical to a differentiated cell or mammal in existence prior to formation of the ICM cell line. Indeed, at the top of page 6144, Sims et al. indicate that they used a 7-8 day embryo to generate the disclosed ICM cell line. This was not a differentiated cell as defined by the present application, which requires at least ten days in culture.

Although Applicants believe that this genetic distinction is clearly recited in the claims by the reference to a differentiated cell nucleus donor in the base method claim, each of the product claims at issue has been amended to clearly indicate that the claimed embryo, fetus, offspring, progeny or cell line has a genotype which is identical to a prior-existing differentiated cell or mammal that was not created itself by nuclear transfer techniques. The Examiner herself has admitted, as discussed

above, that "for this the artisan can only rely on the instant specification" (page 3 of the Office Action). Accordingly, reconsideration and withdrawal of the rejection of Claims 17-19, 25-27, 71-73, 75 and 77, and claims 20-22, 57, 59, 61, 64, 66, 68, 74 and 76 under 35 U.S.C. §102(b) is respectfully requested.

Claim 31 has been rejected under 35 U.S.C. §103(a) as being unpatentable over Sims in view of Lovell-Badge. The Office Action alleges that although Sims does not teach a transgenic ICM cell line, this would have been obvious to do in view of Lovell-Badge, who teach ES cells transfected with a transgene. Applicants respectfully traverse this rejection for the same reasons given above regarding the Sims reference; Claim 31 has also been amended accordingly. Reconsideration and withdrawal of this rejection under 35 U.S.C. §103(a) is therefore respectfully requested in view of the above remarks.

Finally, Claims 33 and 34 have been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Kono et al. The Office Action states that, in the absence of some distinction which indicates a structural or functional difference between the claimed differentiated cells and those taught by Kono et al., Kono allegedly anticipates these claims. Applicants respectfully traverse.

This rejection, is traversed somewhat differently from those discussed above, because differentiated cells that are growing and dividing are genetically identical to each parent cell; therefore, in the case of these claim s, the argument that such

cells would be distinguished as having the identical genotype as a prior-existing differentiated cell does not hold true. Furthermore, isolated primary cells growing in culture as disclosed in Kono et al. should theoretically have the identical genotype as the prior-existing differentiated cell or mammal that was the source of the cell line. However, Applicants may distinguish the claimed cells on another level.

Applicants respectfully first refer to Kono et al., on the last page, where it is admitted that HHY41, and any similar cell line capable of *in vitro* propagation, is transformed or genetically altered to some degree. In hepatocyte cell lines, for instance, this commonly involves a deletion of chromosome 1p (see col. 1, first full paragraph).

The differentiated cells of the present invention, however, are grown and propagated directly from a CICM cell line that is capable of serving as the nuclear donor for a cloned ungulate. Thus, these cells are genetically "normal", and may be distinguished from the cells of Kono on this basis. Although Applicants believe that this distinction is apparent from the claims as they are presently worded, the claims have been amended accordingly.

However, there is another way the differentiated cells of the present invention may be distinguished from the cells of Kono et al., or any other primary cells growing in culture. When cell fusion is used as a means of creating the CICM cell line and

hence the differentiated cells, these cells have the mitochondrial DNA from both the female oocyte and the nuclear donor. Indeed, although, the female oocyte has been enucleated, the mitochondrial DNA has not been removed; hence, both sets of mitochondrial DNA are inherited.

In normal inheritance, mitochondrial DNA is passed down only from the mother, but here, cells with two separate mitochondrial DNAs are achieved. To reflect this distinction, Applicants have introduced two new claims, Claims 78 and 79, which are directed to the differentiated cells of Claims 33 and 34 whereby the parent CICM cell line was produced by cell fusion techniques. The new claims do not contain new matter, and are actually the preferred embodiment of the present invention (see bottom of specification, page 25). The cells of these new claims are clearly differentiated from the cells of Kono et al., because they contain two mitochondrial DNAs. Thus, reconsideration and withdrawal of the rejection of the claims based on Kono et al. is respectfully requested.

This Response is believed to fully address and resolve every issue raised in the Office Action dated December 30, 1998. Accordingly, a Notice of Allowance is believed to be next in order. If the Examiner believes that an interview might help

expedite prosecution of the subject application, she is respectfully requested to telephone the undersigned so that an interview may be arranged.

Respectfully submitted,

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DATE: March 30, 1998